

From: Davis, Minh-Tam
Sent: Wednesday, March 03, 2004 10:23 AM
To: STIC-ILL
Subject: Reprint request for 09/857691

1) Delta-aminolevulinic acid-mediated photosensitization of prostate cell lines: implication for photodynamic therapy of prostate cancer.

Chakrabarti P; Orihuela E; Egger N; Neal D E; Gangula R; Adesokun A; Motamedi M
Department of Surgery, University of Texas Medical Branch, Galveston 77555, USA.

Prostate (UNITED STATES) Sep 1 1998, 36 (4) p211-8, ISSN 0270-4137
Journal Code: 8101368

Contract/Grant No.: NIH-R44CA 60225-02; CA; NCI

2) 0013760372 BIOSIS NO.: 200200353883

A novel murine model of allogeneic vaccination against prostate cancer

AUTHOR: Labarthe Marie-Christine (Reprint); Thraves Peter; Theocharous Pantelli; Dagleish Angus (Reprint); Whelan Mike

AUTHOR ADDRESS: Oncology, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE, UK**UK

JOURNAL: FASEB Journal 16 (4): pA333 March 20, 2002 2002

MEDIUM: print

CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002; 20020420

ISSN: 0892-6638

Thank you.

MINH TAM DAVIS

ART UNIT 1642

ROOM 3A24, MB 3C18

272-8030

Supported by the Kidney Foundation of Canada, FRSQ and FRSQ-FCAR
Santé

246.3

Recombinant *M. smegmatis* secreting IL-15 is efficacious against bladder cancer in vivo.

Sarah Louise Young¹, Michael Murphy², Patricia Hamden², Andrew Mark Jackson¹. ¹Applied immunology Group, St. James's University Hospital, Imperial Cancer Research Fund (UK), Beckett street, Leeds, West Yorkshire LS97TF United Kingdom, ²Imperial Cancer Research Fund (UK), Leeds, West Yorkshire, ³Applied Immunology Group, Imperial Cancer Research Fund (UK), Leeds, West Yorkshire United Kingdom

IL-15 secreting *M. Smegmatis* was assessed for its ability to induce immune responses & protect against tumour challenge. C57Bl/6 mice were given 6 doses of recombinant (r) or wild-type *M. Smegmatis* and co-challenged with MB49 tumour cells.

Mice treated with rM. *Smegmatis*/IL-15 developed small tumours which rapidly resolved permitting the mice to survive in a tumour-free state (>100 days). Lymphocytes from these mice had pronounced antigen-specific proliferative responses & concomitant IFN gamma production. In contrast, mice treated with wild-type bacteria or buffer alone grew large tumours & did not survive (<27 days). Lymphocytes from these mice exhibited low levels of proliferation & showed elevated levels of IL-10 production.

Histopathological examination of the tumour site revealed a significant increase in numbers of CD3+ & non-CD3+ lymphocytes in C57Bl/6 mice treated with rM. *Smegmatis*/IL-15. Furthermore, the anti-tumour immune response generated by this vaccine was shown to be independent of T-lymphocytes, as Nude mice also rejected MB49 tumour cells. We are presently examining the role of these lymphocytes in tumour rejection in response to recombinant mycobacterial vaccines.

246.4

Immunotherapy of Human Follicular Lymphoma: Delineation of T cell Epitopes

Sivasubramanian Baskar¹, Larry W. Kwak². ¹Experimental Transplantation & Immunology, IRSP, SAIC-Frederick, Inc., NCI Frederick, Building 567, Room 203, Frederick, Maryland 21702, ²Experimental Transplantation & Immunology, NCI, Frederick, Maryland

Post-vaccine PBMC from follicular lymphoma patients vaccinated with autologous tumor immunoglobulin (idiotype, Id) exhibited tumor-specific cytotoxicity and cytokine production in vitro (Nature Medicine, 1999, 5:1171). In order to understand the precise nature of antigenic epitope(s) recognized by T cells, we generated T cell lines and T cell clones from patients' post-vaccine PBMC, and demonstrated that these T cells specifically recognize autologous Id as well as autologous tumor in vitro. The T cell responses were measured by the antigen-induced proliferation, intracellular cytokine production and cytokine secretion. Both CD4+ and CD8+ T cells participated in these responses and the responses were MHC restricted. Further studies with synthetic peptides showed that the antigenic epitopes were present in the hypervariable regions (CDR2 and CDR3) of the tumor Id protein. Thus far, no antigenic epitope was identified in the conserved framework regions of the tumor immunoglobulin. Some of these T cells, also recognized Id/tumor cells from other patients who are partially HLA-matched suggesting the possible existence of shared tumor antigens among the malignant B cells from different individuals.

246.5

Analysis of the Antitumor Effect of Different Her-2/neu-expressing Plasmid DNAs in a Syngeneic Tumor Model.

Joon Youb Lee¹, Dong-Hyeon Kim¹, Yeonseok Chung¹, Seung-Uon Shin², Chang-Yuil Kang¹. ¹College of Pharmacy, Seoul National University, Shillimdong, Kwanakgu, Seoul 151-742 Korea, Republic of, ²Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida

Four Her-2/neu-expressing plasmids (pNeuTM, pNeuTM-gDs, pNeuECD, and pNeuECD-gDs) were generated encoding either transmembrane or secreted human Her-2/neu. While pNeuTM and pNeuECD encode the original signal peptide sequence, the signal peptide sequence of pNeuTM-gDs and pNeuECD-gDs was replaced by the signal peptide sequence from glycoprotein D of herpes simplex virus type I. We examined whether i.m. injection of either of these plasmids could induce CTL and antibody against Her-2/neu in BALB/C mice. All plasmids induced strong CTL activity. pNeuTM and pNeuECD induced high Her-2/neu-specific serum IgG titers and pNeuTM-gDs and pNeuECD-gDs induced low or undetected serum IgG titers. To evaluate the antitumor effect of these plasmids, we

immunized BALB/C mice with either of the plasmids before Her-2/neu-expressing tumor cells were injected either subcutaneously or intravenously. As a result, mice vaccinated with not only pNeuTM and pNeuECD but also pNeuTM-gDs and pNeuECD-gDs exhibited complete eradication of tumor cells. These studies demonstrate that CTL may be sufficient in rejecting Her-2/neu-expressing tumor cells.

246.6

A novel murine model of allogeneic vaccination against prostate cancer.

Marie-Christine Labarthe¹, Peter Thraves², Pantelli Theocharous², Angus Dalglish¹, Mike Whelan². ¹Oncology, St. George's Hospital Medical School, Cranmer Terrace, London, London SW17 0RE United Kingdom, ²Onyvox Ltd, London, London United Kingdom

The development of allogeneic whole cell vaccines for prostate cancer is complicated by the lack of a relevant animal model. The murine system is most attractive as it allows for high throughput and allogenicity can easily be investigated. However, the lack of murine prostatic lines has hampered development.

The prostate was removed from a male C3H (H-2k) mouse and cells transformed with an E6/E7 construct from HPV-18. The resulting line (PMC-1) was positive for cytokeratin 18, vimentin and androgen receptor and negative for desmin by immunohistochemistry. Androgen receptor was upregulated by culturing the cells in testosterone. Flow cytometry revealed a high level of MHC I expression, as well as CD80 and CD54. PMC-1 did not form tumours in nude mice.

Female C57 (H-2b) mice were vaccinated with irradiated PMC-1 subcutaneously and challenged with syngeneic prostate tumour cell line RM9. Vaccinated animals showed a clear survival benefit.

Initial investigation revealed high levels of NK activity and low CTL activity in vaccinated mice. This is in marked contrast to the murine B16 melanoma model and may suggest that T-cell activation is difficult to achieve in the prostate.

246.7

PRECLINICAL CANCER VACCINE STUDIES IN MICE USING A HER-2 PEPTIDE IMMUNOGEN COMBINED WITH THE SAPONIN-BASED IMMUNE ENHANCER GPI-0100 AND POLYSACCHARIDES

Richard D. May¹, Pierre L. Triozzi², Wayne A. Aldrich², Robert C. Reynolds³, Ashish K. Pathak³, Dante J. Marciani⁴. ¹Cancer Therapeutics and Immunology, Southern Research Institute, 2000 Ninth Avenue South, Birmingham, AL 35205, ²Oncology/Hematology, University of Alabama at Birmingham, Birmingham, AL, ³Organic Chemistry, Southern Research Institute, Birmingham, AL, ⁴Galenica Pharmaceuticals, Inc., Birmingham, AL

HER-2 is over-expressed by many common malignancies and is an attractive target antigen for vaccine approaches. An oligopeptide immunogen targeting a B-cell epitope of the HER-2 extracellular domain plus a measles virus T-helper epitope to augment immunogenicity was constructed. This chimeric immunogen, MVF-HER-2 (628-647), elicits HER-2-specific antibody with antitumor activity in mice. Oligopeptides are relatively weak immunogens in humans. Thus, immune enhancers (adjuvants) are needed for oligopeptide immunogens. Preclinical studies were conducted in mice to examine the immune responses of MVF-HER-2 combined with GPI-0100, a water-soluble, semi-synthetic triterpenoid saponin immune enhancer. To possibly further enhance activity, GPI-0100 was combined with two polysaccharides and their chemically-modified derivatives. These polysaccharides, pectin and carboxy- β -glucan, are both immunologically active. Various formulations were used to immunize mice (2 biweekly s.c. injections). Controls were vehicle only (PBS) and MVF-HER-2 only. Two weeks after the second immunization, sera were tested for the antibody response by ELISA and spleen cells were tested for their in vitro antigen-specific T cell proliferative responses. Significant antibody and lymphoproliferative responses were induced. The results indicate that MVF-HER-2 and GPI-0100 (with or without polysaccharides) will make an effective cancer vaccine in upcoming clinical trials.

STIC-ILL

NPL
Q44301 F4

From: Davis, Minh-Tam
Sent: Wednesday, March 03, 2004 10:23 AM
To: STIC-ILL
Subject: Reprint request for 09/857691

1) Delta-aminolevulinic acid-mediated photosensitization of prostate cell lines: implication for photodynamic therapy of prostate cancer.

Chakrabarti P; Orihuela E; Egger N; Neal D E; Gangula R; Adesokun A; Motamedi M
Department of Surgery, University of Texas Medical Branch, Galveston 77555, USA.

Prostate (UNITED STATES) Sep 1 1998, 36 (4) p211-8, ISSN 0270-4137
Journal Code: 8101368

Contract/Grant No.: NIH-R44CA 60225-02; CA; NCI

2) 0013760372 BIOSIS NO.: 200200353883

A novel murine model of allogeneic vaccination against prostate cancer

AUTHOR: Labarthe Marie-Christine (Reprint); Thraves Peter; Theocharous Pantelli; Dagleish Angus (Reprint); Whelan Mike

AUTHOR ADDRESS: Oncology, St. George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE, UK**UK

JOURNAL: FASEB Journal 16 (4): pA333 March 20, 2002 2002

MEDIUM: print

CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists on Experimental Biology New Orleans, Louisiana, USA April 20-24, 2002; 20020420

ISSN: 0892-6638

Thank you.

MINH TAM DAVIS

ART UNIT 1642

ROOM 3A24, MB 3C18

272-8030

Delta-Aminolevulinic Acid-Mediated Photosensitization of Prostate Cell Lines: Implication for Photodynamic Therapy of Prostate Cancer

Pradip Chakrabarti,^{1,2} Eduardo Orihuela,^{1*} Norman Egger,³
Durwood E. Neal Jr.,¹ Rama Gangula,¹ Adekunle Adesokun,⁴ and
Massoud Motamedi^{1,2}

¹*Division of Urology, Department of Surgery, University of Texas Medical Branch,
Galveston, Texas*

²*Biomedical Laser and Spectroscopy Program, Biomedical Engineering Center, University of
Texas Medical Branch, Galveston, Texas*

³*Department of Preventive Medicine and Community Health, University of Texas Medical
Branch, Galveston, Texas*

⁴*Department of Pathology, University of Texas Medical Branch, Galveston, Texas*

BACKGROUND. Delta-aminolevulinic acid (ALA)-mediated photodynamic therapy (PDT) is currently being investigated for the treatment of prostate diseases. In this study, we evaluate 1) the in vitro production of protoporphyrin IX (PPIX) (the active photosensitizing agent of ALA-mediated PDT) by two different prostate cancer cell lines (LNCaP and PC-3) and a benign, modified, prostatic cell line (TP-2), and 2) the extent of PDT-induced cell injury, as determined by electron microscopy (EM) and cell survival.

METHODS. The cell lines were assigned into four treatment groups: group 1, control, no ALA and no light irradiation; group 2, dark control, ALA only; group 3, light control, radiation only; and group 4, PDT, ALA followed by irradiation (630 nm, 3 joules/cm²). The experiments were performed in triplicate. ALA concentration was 50 µg/ml of media in all instances.

RESULTS. Following incubation with ALA, PPIX production was significantly increased in the three cell lines studied, and more notably in the PC-3 cell line. Compared to controls, EM and cell survival studies demonstrated significant mitochondrial damage and decreased survival, respectively, in the cells treated with PDT. This was also more evident in the PC-3 cell line.

CONCLUSIONS. Our results demonstrate that prostate cells differ in their response to ALA-mediated PDT. This response appears to depend on the intracellular production of PPIX and the cell type, i.e., on the functional and structural characteristics of the cell mitochondria. In

Part of this study was presented as a poster at the 1997 fall symposium of the Society of Basic Urologic Research, Pacific Grove, CA. Contract grant sponsor: US Department of Energy; Contract grant number: DE-FJ 03-95 ER 61971; Contract grant sponsor: National Institute of Health; Contract grant number: NIH-R44CA 60225-02.

*Correspondence to: Eduardo Orihuela, M.D., Professor of Surgery and Radiology, Section Head, Urologic Oncology, John Sealy Annex, Route 0540, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555.

Received 28 July 1997; Accepted 9 April 1998

addition, our results suggest that PDT might be effective at killing prostate cancer cells. *Prostate* 36:211–218, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: photodynamic therapy; delta-aminolevulinic acid; prostate cancer; PC-3; LNCaP; TP-2 cell lines

INTRODUCTION

Several studies have shown the potential of photodynamic therapy (PDT) as a treatment modality for various malignancies [1–6]. PDT entails the activation of a photosensitizing agent within cells or tissues by light in the presence of oxygen. Oxygen molecules are converted into various reactive oxygen species, including "singlets" [7]. Singlet oxygen is a highly toxic substance that is known to react with many biologically important structures, including subcellular organelles, proteins, and nucleic acids [8]. Several classes of compounds have shown promise as photosensitizing agents, of which porphyrins constitute the fraction most widely studied. Delta-aminolevulinic acid (ALA) is not a porphyrin; nevertheless, it is endogenously converted to protoporphyrin IX (PPIX), which is a porphyrin that acts as a photosensitizing agent. ALA-mediated photosensitization is widely studied because of its potential advantages over other photosensitizing agents, such as the rapid elimination of PPIX from the body (within 12–24 hr), thus reducing the problem of phototoxicity commonly encountered with other agents [9]. ALA is formed within the cell by the combination of a molecule of glycine with a molecule of succinyl Co-A, by the action of the enzyme ALA synthase in the heme pathway [10]. The action of this enzyme, ALA-synthase, is a rate-limiting step in the heme biosynthetic pathway [10]. However, high levels of PPIX can be achieved by administering exogenous ALA, thus bypassing the rate-limiting step dependent upon the enzyme ALA-synthase [11]. This phenomenon, which appears to be enhanced in malignant cells [11,12], is potentially exploitable from the viewpoint of PDT.

We have previously shown, in the canine animal model, that high concentrations of PPIX can be selectively achieved in the prostate following intravenous administration of ALA [13,14]. In that animal model, PDT of the prostate, irradiated transurethrally after IV administration of ALA, produced lesions which were characterized by interstitial hemorrhage, intravascular thrombosis, and coagulation necrosis immediately after PDT was delivered, and later on, by glandular atrophy associated with squamous metaplasia [13]. In addition, PDT of the canine prostate with other photosensitizing agents has also been shown by various investigators to cause photodestruction of prostatic tissue [15,16]. Further, a recent study has demon-

strated that apoptosis is induced in carcinoma cell lines, including the PC-3 cell line (one of the cell lines used in our study), following PDT using photofrin as the photosensitizing agent [17].

The above results encouraged us to investigate the possibility of ALA-mediated PDT for prostate cancer. Prostate cancer cells, as in other solid tumors, are heterogeneous, and little is known about the effect of ALA-mediated PDT on the different types of cells that constitute prostate cancer. The objectives of our study were: 1) to evaluate if ALA-mediated PDT has significant effect on prostate cancer cells, and 2) to determine if cell type influences the PDT effect. In order to appraise our objectives, we determined the ability of two different prostate cancer cell lines and one modified benign prostate epithelial cell line to produce PPIX following incubation with ALA. Second, we assessed, by electron microscopy (EM), the extent of cell injury following ALA-mediated PDT of the different cell lines. Third, we estimated cell survival in the cell lines following ALA-mediated PDT.

MATERIALS AND METHODS

Cell Lines and Culture

PC-3, an anaplastic human prostate carcinoma cell line [18,19], and LNCaP, a well-differentiated prostatic carcinoma cell line [20,21], were obtained from the American Type Culture Collection (ATCC, Rockville, MD). TP-2, a benign prostatic epithelial cell line immortalized by transfection with a retrovirus [22,23], was supplied by William Isaacs, (James Buchanan Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD). These cell lines were chosen because they approximate benign prostatic epithelial cells and well-differentiated and anaplastic prostate cancer.

LNCaP cells were grown in RPMI-1640 with L-glutamine and 10% fetal bovine serum (Mediatech, Fisher Scientific, Houston, TX). PC-3 cells were grown in F12K medium (Gibco, Grand Island, NY) supplemented with 7% fetal bovine serum. TP-2 cells were cultured in MCDB 153 and RPMI-1640 in a 1:1 ratio supplemented with 5% fetal bovine serum, 5 mg/ml insulin, 250 nM/ml dexamethasone, 10 ng/ml cholera toxin, 35 mg/ml bovine pituitary extract, 100 u/ml penicillin, and 50 mg/ml streptomycin. All cell cultures were incubated at 37°C with 5% CO₂.

Measurement of Intracellular PPIX Concentration Following Incubation With ALA

Delta-aminolevulinic acid (Sigma Chemical Co., St. Louis, MO) was dissolved in phosphate-buffered saline (PBS) solution and added to the cell cultures at a concentration of 50 mg/ml of media, which is comparable with the serum concentration of ALA used in clinical studies [24,25]. Cell cultures were incubated with ALA for 2, 4, 6, and 24 hr.

Cells were harvested from cultures incubated with ALA and from controls (cultures incubated without ALA) by trypsinization (0.25% trypsin and 0.1% EDTA). The suspension was centrifuged at 1,250 rpm for 5 min at 20°C and pelleted. The net weight of the pellet was measured. The pellet was then sonicated in 1 ml 0.25 M sucrose-0.02 M Tris buffer (pH 7.4) with a Labsonic/Braun-Sonic 2000U generator (Ultrasonic Power Corp., Freeport, IL) at a frequency of 20 kHz. Porphyrins were extracted by diluting the homogenates 2:5 with 1 M perchloric acid: methanol (1:1 by volume). Homogenates were then centrifuged (10,000 rpm for 3 min), and porphyrin concentration of the supernatant was measured by fluorescence spectrometry using a SLM 8000 C spectrofluorometer (Spectronic Instruments, Inc., Rochester, NY). The excitation wavelength was 400 nm and the fluorescence emission peak was recorded at 650 nm [26]. A coproporphyrin III solution (0.5 mg/ml in 1N-HCl) was used as a fluorescence standard, and the protoporphyrin concentration was calculated using a previously determined fluorescence ratio of coproporphyrin III to protoporphyrin IX under measurement conditions. These experiments were performed in triplicate for each cell line at each time point. The aforesaid method was also applied prior to the administration of ALA, to rule out the presence of detectable amounts of PPIX in the culture media of each cell line.

Photodynamic Therapy

Cells were assigned into four treatment groups: group 1 (controls), no ALA, no irradiation; group 2 (dark controls), ALA only (no irradiation); group 3 (light control), irradiation only (no ALA); and group 4 (PDT), ALA followed by and irradiation.

Group 1 was directly processed for EM studies. In group 2, the cells were incubated with ALA (50 µg/ml media) for 4 hr and then harvested and processed for EM. In group 3, the cells were irradiated only and harvested immediately for EM. In group 4, cell lines were incubated with ALA for 4 hr and irradiated, harvested, and processed for EM. Irradiation was applied with laser light at 3 joules/cm² (15 mW/cm² × 200 sec) from an argon-pumped dye laser (Spectra Physics La-

sers, Inc., Mountain View, CA) emitting 631 nm radiation. The laser beam was delivered via a 400-mm (core diameter) hard-clad silica fiber with a divergence angle of the fiber of 12° (3M Specialty Optical Fibers, West Haven, CT). The cells were harvested by trypsinization and pelleted.

Electron Microscopy

The cell pellets were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer for at least 4 hr, and then postfixed in 1% osmium tetroxide, and stained en bloc by 2% uranyl acetate. The specimen was dehydrated by graded ethanol, and embedded in polybed/812 resin. Both thick and thin sections were cut on a Sorvall MT-6000 ultramicrotome, and 70-nm thin sections were stained by modified Reynold lead citrate, and examined under the Philips 201 electron microscope.

Cell Survival

Cell survival was determined 1) following 2, 4, and 6 hr of incubation with ALA (dark toxicity), and 2) 24 hr after ALA-mediated PDT. These cells had been incubated with ALA for 4 hr prior to PDT. A colorimetric, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) assay technique was adopted [27]. Into selected wells of a 96-well, flat-bottom tissue culture plate, containing 0.1 ml culture media, 0.01 ml MTT solution was added and mixed by gently tapping the side of the tray. The tray was then incubated at 37°C for 4 hr. Then, 0.1 ml isopropanol was added to the selected wells and after 15 min, the absorbance was measured on an ELISA plate reader (2550, Bio-RAD) using a 575-nm bandpass filter. Absorbance of the solution from the treated cell plates was divided by that of the control cell plates (untreated cells) to derive the fraction of survival. These experiments were performed in triplicate.

Statistical Analysis

Data are presented as mean ± SD. Increases of PPIX levels compared to baseline were assessed by repeated-measures analysis of variance in each cell line. Differences of PPIX levels at all time points between cell lines were compared by analysis of variance. If analysis of variance indicated significant differences, the Bonferroni procedure was applied [28]. The effects of PDT on cell survival, compared to the corresponding light controls, were analysed by unpaired two-tailed *t*-test. Significance was determined at *P* < 0.05.

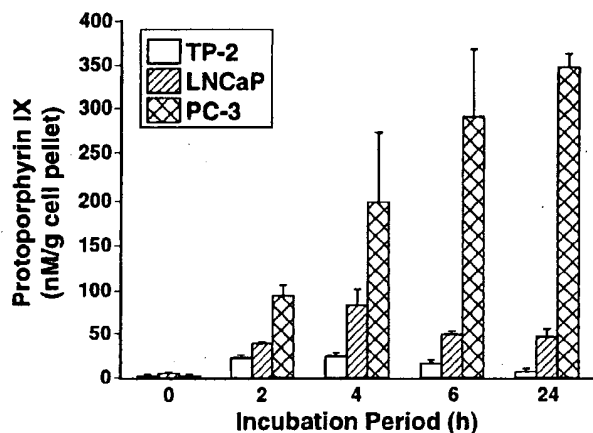


Fig. 1. Mean concentration \pm SD of protoporphyrin IX before (0 hr) and after incubation with ALA (50 μ g/ml).

RESULTS

Concentration of PPIX Following Incubation With ALA

The three cell lines showed marked increases in their concentration of PPIX after incubation with ALA when compared to baseline ($t = 0$ hr). This increase was statistically significant for the PC-3 cell line ($P = 0.0045$), for the LNCaP cell line ($P = 0.0032$), and for the TP-2 cell line ($P = 0.0078$). The PC-3 cell line showed the highest concentration of PPIX at all time points compared to the LNCaP cell line ($P < 0.0001$) and to the TP-2 cell line ($P < 0.0001$) (Fig. 1).

Electron Microscopy Following PDT

The control cell lines (group 1) showed differences in the structure of their mitochondria. The mitochondria of PC-3 cells showed only occasional intramitochondrial tubulo-vesicular architecture, which was a highly prominent feature of mitochondria of LNCaP cells and was less prominent in the TP-2 cells (Fig. 2a-c). Cell lines treated with ALA only (group 2), or irradiated with light without incubation with ALA (group 3), appeared similar to their controls (group 1). In contrast, cells incubated with ALA followed by PDT (group 4) revealed extensive mitochondrial injury in the PC-3 cells (Fig. 3a). This injury was not so evident in the other cell lines (Fig. 3b,c).

Cell Survival

Group 1 (controls) and group 2 (dark toxicity). Cell survival at 0, 2, 4, 6, and 24 hr was $\geq 80\%$ in all cell lines at all the time points in both treatment groups.

Group 3 (light control) and group 4 (PDT). Following PDT, significant reduction in survival was observed in the PC-3 and LNCaP cell lines as compared to their corresponding light controls ($P = 0.0002$ and $P = 0.0011$, respectively), whereas PDT had no significant effect on survival of the TP-2 cell line (Fig. 4).

DISCUSSION

Several factors need to be considered in order to understand the significance of differences in the production of PPIX and how this variable affects mitochondrial injury and cell survival following ALA-mediated PDT in the different cell lines studied. Mitochondria occupy 15–50% of the total cytoplasmic volume of most animal cells, and participate in more metabolic functions than any other organelle, and their proper function is essential to maintain normal cellular metabolism [29]. Mitochondria have been implicated as an important target of porphyrin-mediated PDT [30–32]. Our study provides further support to this concept. PPIX is synthesized from ALA in the heme-biosynthesis pathway within the mitochondria [33], and PPIX cannot be produced in cells devoid of mitochondria, i.e., erythrocytes [34]. In addition, some porphyrins are lipophilic compounds that may concentrate selectively in the mitochondria [30–32,35]. This selectivity appears also to be related to the fact that mitochondria have specific mitochondrial-porphyrin-receptors [36]. Further evidence supporting that mitochondria are targets of porphyrin-mediated PDT is provided by several studies that have shown functional impairment of a number of mitochondrial enzymes involved in the oxidative phosphorylation pathway [30–32,35,37,38]. In addition, and in accordance with our findings, hydropic degeneration of the mitochondria following porphyrin-induced PDT has also been observed previously in various animal and human cell lines [11]. In this regard, mitochondrial damage, as determined by EM studies [11] or by enzymatic assay [32], has been shown to directly correlate with cell necrosis. Our results support, at least in part, this contention.

We only observed significant mitochondrial damage and decreased cell survival in cells treated with PDT, which suggests that ALA-mediated PDT might be effective at killing prostatic cancer cells. In addition, our findings also suggest that cell response to PDT is variable. Significant mitochondrial damage and decreased cell survival were present in the PC-3 cells, whereas neither of these findings was noticeable in the TP-2 cells. Furthermore, the LNCaP cells had a mixed response, i.e., their survival was also significantly decreased but their mitochondrial damage, as assessed by EM, was only mild.

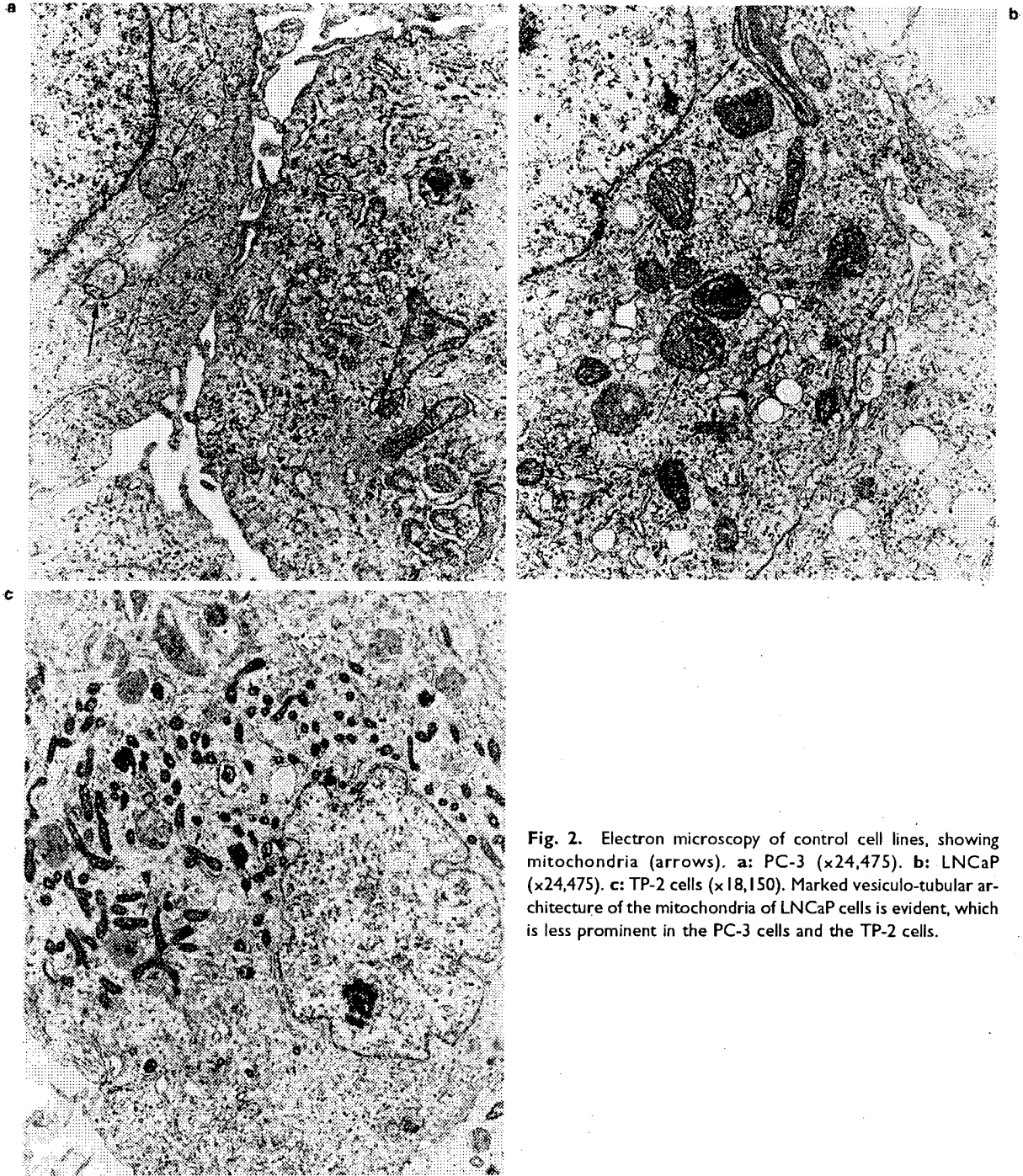


Fig. 2. Electron microscopy of control cell lines, showing mitochondria (arrows). **a:** PC-3 ($\times 24,475$). **b:** LNCaP ($\times 24,475$). **c:** TP-2 cells ($\times 18,150$). Marked vesiculo-tubular architecture of the mitochondria of LNCaP cells is evident, which is less prominent in the PC-3 cells and the TP-2 cells.

Differences in structural and functional characteristics of mitochondria may be one factor accounting for differences in susceptibility to PDT among different cell lines. Mitochondria of tumor cells frequently differ both structurally and functionally from mitochondria isolated from normal cells, and the extent of the disparity correlates with the growth rate or degree

of differentiation of the tumor [39], i.e., smaller, electron-dense mitochondria display higher levels of oxidative phosphorylation when compared to larger, sparsely staining mitochondria [40]. Mitochondria from tumor cells have also been shown to display abnormal shapes, cristae, helical structures, or inclusions that are not present in normal cells [39,41,42]. In ad-

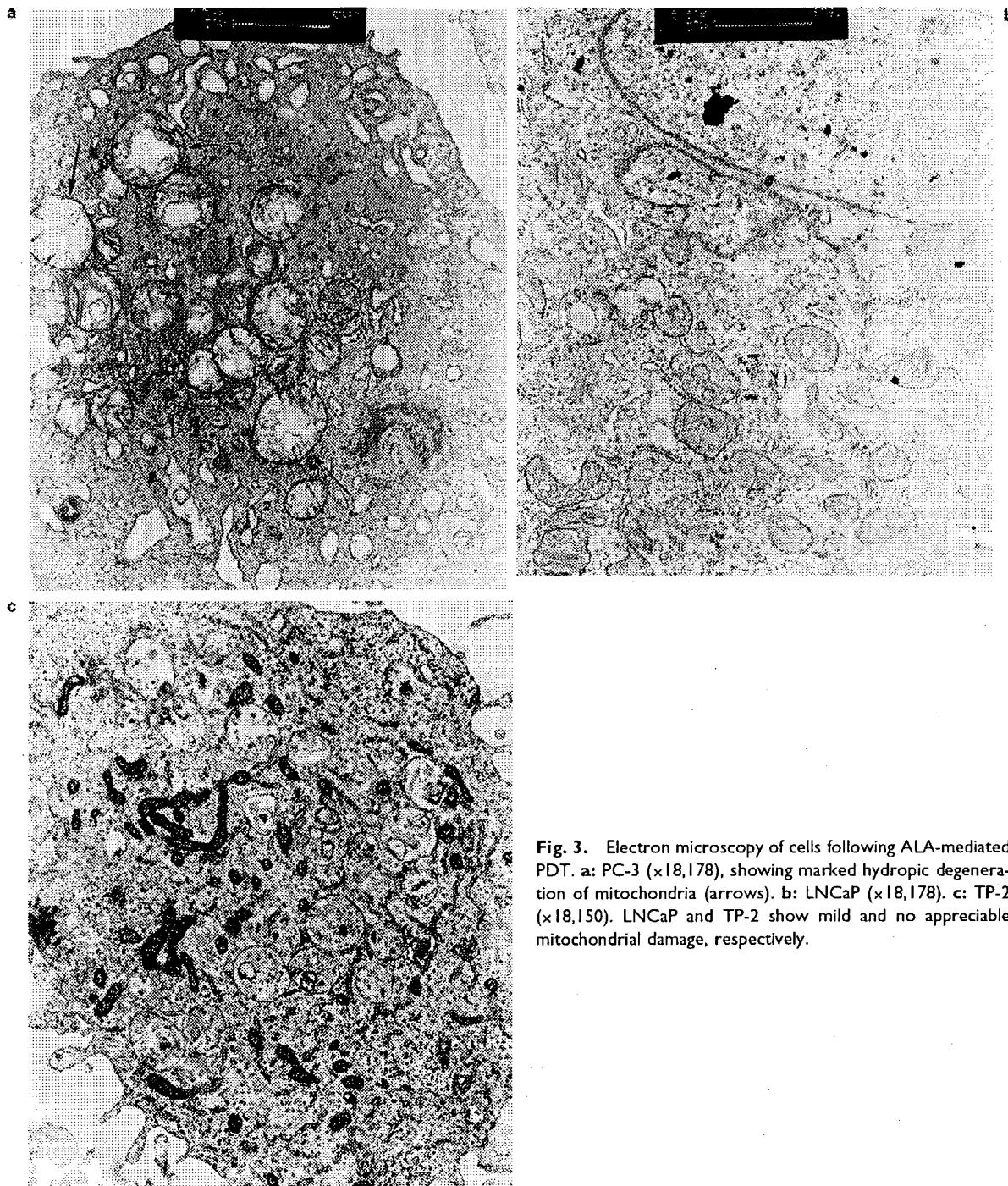


Fig. 3. Electron microscopy of cells following ALA-mediated PDT. **a:** PC-3 ($\times 18,178$), showing marked hydropic degeneration of mitochondria (arrows). **b:** LNCaP ($\times 18,178$). **c:** TP-2 ($\times 18,150$). LNCaP and TP-2 show mild and no appreciable mitochondrial damage, respectively.

dition, rapidly growing tumors tend to have smaller mitochondria with fewer cristae as compared to slowly growing tumors or normal cells [39,41,42]. Our study also supports this concept, i.e., the PC-3 cell mitochondria were different structurally as compared to those of LNCaP and TP-2 cells, and, following PDT, extensive mitochondrial damage was evident only in

the PC-3 cell line. Of course, an important consideration is that differences in the production of PPIX are not necessarily related to differences in mitochondrial structure alone, but to differences in mitochondrial enzymes in the biosynthesis pathway [43,44]. Other investigators have reported a correlation between cell proliferation rates and PPIX synthesis [11]. In our

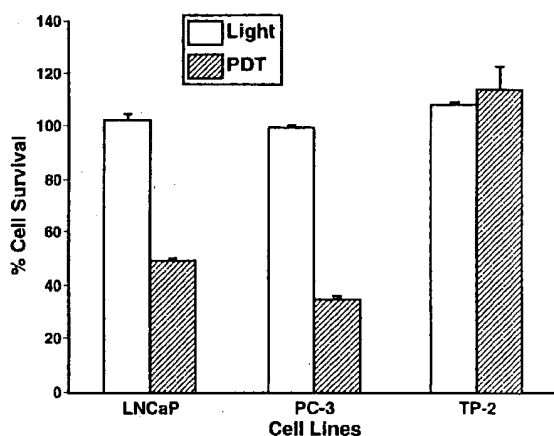


Fig. 4. Cell survival at 24 hr following irradiation only (light control, group 3), and after cells were incubated with ALA followed by irradiation (PDT, group 4).

study, we did not evaluate the mitochondrial enzymatic activity of each cell line studied, and thus we do not know the possible impact of this variable on our results.

Other factors might also account for the differences in the extent of mitochondrial damage that we observed among the cell lines after PDT. The PDT effect depends upon the combination of the amount of photosensitizer in the target at the time of treatment, the amount of light delivered, and the amount of oxygen (O_2) available [45–48]. In our study we did not assess the impact of various concentrations of ALA (or PPIX) on the effect of PDT in the different cell lines studied; nor did we measure the intracellular concentration of ALA. Nonetheless, we observed that the intracellular concentration of PPIX was significantly higher and that the PDT effect was markedly more intense in the PC-3 cell line. In agreement with our findings, other investigators have reported that there appears to be a threshold of intracellular PPIX for PDT-induced cell killing [11], and a direct correlation between PPIX concentration and PDT effect [9].

It is worth mentioning that we previously reported that a high concentration of PPIX can be obtained in the *in vivo* canine prostate [14], and that significant lesions are induced in this model following light exposure [13], which is in contrast to the lack of PDT effect in the TP-2 cells in this study. This disparity is due, at least in part, to differences in the ALA pharmacokinetics between *in vitro* and *in vivo* models, as well as differences in the radiation dose. More important, perhaps, is that the response to PDT in the canine model was also related to stromal and vascular injury, characterized by intravascular thrombosis and interstitial hemorrhage, that is associated with ischemia and coagulation necrosis [49].

CONCLUSIONS

In summary, our study suggests that ALA-mediated PDT might be effective at killing prostatic cancer cells. The PDT effect is variable and appears to depend, at least in part, on functional and perhaps structural characteristics of mitochondria of the target cells and on the concentration of PPIX. Mechanisms that affect the uptake of ALA, the production of PPIX, mitochondrial sensitivity to oxidative injury, and the capability of cells to repair mitochondrial damage are probably all relevant and have significant impact on ALA-mediated PDT. How these variables influence the effects of ALA-mediated PDT of prostate cancer has yet to be determined.

ACKNOWLEDGMENTS

We thank Dr. William Isaacs of the James Buchanan Brady Urological Institute, Johns Hopkins Hospital (Baltimore, MD), for kindly supplying the TP-2 cell line.

REFERENCES

- Kessel D: Photodynamic therapy and neoplastic disease. *Oncol Res* 1992;4:219–225.
- Dougherty T, Marcus S: Photodynamic therapy. *Eur J Cancer* 1992;28:1734–1742.
- Hillegersberg V, Kort W, Wilson J: Current status of photodynamic therapy in oncology. *Drugs* 1994;48:510–527.
- Fisher A, Murphree A, Gomer C: Clinical and preclinical photodynamic therapy. *Lasers Surg Med* 1995;17:2–31.
- Lee L, Whitehurst C, Chen Q, Plantelides M, Hetzel F, Moore J: Interstitial photodynamic therapy in the canine prostate. *Br J Urol* 1997;80:898–902.
- Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky KE, Nesland JM: 5-aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. *Cancer* 1997; 79:2282–2308.
- Kessel D: Effects of photoactivated porphyrins at the cell surface of leukemia L1210 cells. *Biochemistry* 1977;16:3443–3449.
- Weishaup KR, Gomer CJ, Dougherty TJ: Identification of singlet oxygen as the cytotoxic agent in photo-inactivation of a murine tumor. *Cancer Res* 1976;36:2326–2329.
- Kennedy JC, Pottier RH: Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. *J Photochem Photobiol B* 1992;14:275–292.
- Murray RK: Porphyrins and bile pigments. In Murray RK, Granner DK, Mayes PA, Rodwell VW (eds): "Harper's Biochemistry," 22nd ed. Norwalk, CT/San Mateo, CA: Appleton and Lange, 1990:318–331.
- Iinuma S, Farshi SS, Ortel B, Hasan T: A mechanistic study of cellular photodestruction with 5-aminolevulinic acid-induced porphyrin. *Br J Cancer* 1994;70:21–28.
- Zhongxue H, Gibson SL, Foster TH, Hilf R: Effectiveness of d-aminolevulinic acid-induced protoporphyrin as a photosensitizer for photodynamic therapy *in vivo*. *Cancer Res* 1995;55: 1723–1731.
- Johnson S, Motamedi M, Egger N, Pow-Sang M, Orihuela E, Anderson K, Warren MM: Photosensitizing the canine prostate

- with 5-aminolevulinic acid: A new laser prostatectomy? *J Urol* 1995;153:298.
14. Egger N, Motamedi M, Pow-Sang M, Orihuela E, Anderson KE: Accumulation of porphyrins in plasma and tissues of dogs after d-aminolevulinic acid administration: Implications for photodynamic therapy. *Pharmacology* 1996;52:362-370.
 15. Shetty SD, Sirls LT, Chen Q, Hetzel F, Cerny JC: Interstitial photodynamic therapy for the prostate: A canine feasibility study. *J Urol* 1995;153:415.
 16. Selman SH, Keck RW: The effect of transurethral light on the canine prostate after sensitization with the photosensitizer tin (II) etiopurpurin dichloride: A pilot study. *J Urol* 1994;152:2129-2132.
 17. He XY, Sikes RA, Thomsen S, Chung LWK, Jacques SL: Photodynamic therapy with photofrin II induces programmed cell death in carcinoma cell lines. *Photochem Photobiol* 1994;59:468-473.
 18. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* 1979;17:16-23.
 19. Ohnuki Y, Marnell MM, Babcock MS, Lechner JF, Kaighn ME: Chromosomal analysis of human prostatic adenocarcinoma cell lines. *Cancer Res* 1980;40:524-534.
 20. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Ming Chu T, Miranda EA, Murphy GP: LNCaP model of human prostatic carcinoma. *Cancer Res* 1983;43:1809-1818.
 21. Gibas Z, Becher R, Kawinski E, Horoszewicz J, Sandberg AA: A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). *Cancer Genet Cytogenet* 1984;11:399-404.
 22. Sanda MG, Nicholas PR, Walsh JC, Kawakami Y, Nelson WG, Pardoll DM, Simons JW: Molecular characterization of defective antigen processing in human prostate cancer. *J Natl Cancer Inst* 1995;87:280-285.
 23. Isaacs WB: Immortalization of normal human prostate epithelial cells by a retrovirus expressing the adenovirus E1A gene. *J Urol* 1992;147:415.
 24. Milkvy P, Messmann H, Regula J, Conio M, Pauer M, Millson CE, MacRobert AJ, Bown SG: Sensitization and photodynamic therapy (PDT) of gastrointestinal tumors with 5-aminolaevulinic acid (ALA) induced protoporphyrin IX (PPIX). A pilot study. *Neoplasia* 1995;42:109-113.
 25. Regula J, MacRobert AJ, Gorchin A, Buonaccorsi GA, Thorpe SM, Spencer GM, Hatfield AR, Bown SG: Photosensitization and photodynamic therapy of esophageal, duodenal, and colorectal tumors using 5 aminolaevulinic acid induced protoporphyrin IX—A pilot study. *Gut* 1995;36:67-75.
 26. Anderson KE: Effects of antihypertensive drugs on hepatic heme biosynthesis, and evaluation of ferrochelatase inhibitors to simplify testing of drugs for heme pathway induction. *Biochim Biophys Acta* 1978;543:313-327.
 27. Mosmann T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. *J Immunol Methods* 1983;65:55-63.
 28. Dawson-Saunders, B: The Bonferroni procedure. In Dawson-Saunders B, Trapp RG (eds): "Basic and Clinical Biostatistics," 2nd ed. Norwalk, CT: Appleton and Lange, 1994:134-142.
 29. Modica-Napolitano JS, Steele GD Jr, Chen LB: Aberrant mitochondria in two human colon carcinoma cell lines. *Cancer Res* 1989;49:3369-3373.
 30. Hilf R, Gibson SL, Penney DP, Ceckler TL, Bryant RG: Early biochemical responses to photodynamic therapy monitored by NMR spectroscopy. *Photochem Photobiol* 1987;46:809-817.
 31. Boegheim JJP, Lagerberg JWM, Dubbleman TMAR, Tijssen K, Tanke HJ, Van der Meulen J, Van Steveninck J: Photodynamic effect of hematoporphyrin derivative on the uptake of Rh 123 by mitochondria of intact murine L929 fibroblasts and Chinese hamster ovary K1 cells. *Photochem Photobiol* 1988;48:613-620.
 32. Salet C, Moreno G: New trends in photobiology (invited review): Photosensitization of mitochondria. Molecular and chemical aspects. *J Photochem Photobiol B* 1990;5:133-150.
 33. Abels C, Heil P, Dellian M, Kuhnle GEH, Baumgartner R, Goetz AE: In vivo kinetics and spectra of 5-aminolaevulinic acid-induced fluorescence in an amelanotic melanoma of the hamster. *Br J Cancer* 1994;70:826-833.
 34. Sassa S: Synthesis of heme. In Williams WJ, Bentler E, Erslev AJ, Lichtman MA (eds): "Hematology," 4th ed. New York: McGraw-Hill, 1990:322-329.
 35. Woodburn KW, Vardaxis NJ, Kaye AA, Reiss JA, Phillips D: Evaluation of porphyrin characteristics required for photodynamic therapy. *Photochem Photobiol* 1992;55:697-704.
 36. Verma A, Nye J, Snyder S: Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. *Proc Natl Acad Sci USA* 1987;84:2256-2260.
 37. Moreno G, Salet C: Cytotoxic effects following micro-irradiation of cultured cells sensitized with hematoporphyrin derivative. *Int J Radiat Biol* 1985;47:383-387.
 38. Hilf R, Murrant RS, Narayanan U, Gibson SL: Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors. *Cancer Res* 1986;46:211-217.
 39. Pedersen PL: Tumor mitochondria and the bioenergetics of cancer cells. *Prog Exp Tumor Res* 1978;22:190-274.
 40. Hackenbrock CR: Ultrastructural bases for metabolically linked mechanical activity in mitochondria. *J Cell Biol* 1968;37:345-369.
 41. Hruban Z, Mochizuki Y, Slesers A, Morris HP: A comparative study of cellular organelles of Morris hepatomas. *Cancer Res* 1972;32:853-867.
 42. Hruban Z, Mochizuki Y, Morris HP, Slesers A: Ultrastructure of Morris renal tumors. *J Natl Cancer Inst* 1973;50:1487-1495.
 43. Pedersen PL, Greenwalt JW, Chan TL, Morris HP: A comparison of some ultrastructural and biochemical properties of mitochondria from Morris hepatomas 9618A, 7800, and 3294A. *Cancer Res* 1970;30:2620-2626.
 44. Van Hillegersberg R, Van Den Berg JWO, Kort WJ, Terpstra OT, Wilson JHP: Selective accumulation of endogenously produced porphyrins in a liver metastasis model in rats. *Gastroenterology* 1992;103:647-651.
 45. Dailey HA, Smith A: Differential interaction of porphyrins used in photoradiation therapy with ferrochelatase. *J Biochem (Tokyo)* 1984;223:441-445.
 46. Profio AE, Doiron DR: Dosimetry considerations in phototherapy. *Med Phys* 1981;8:190-196.
 47. Berns MW, Berns GS, Coffey J, Wile AJ: Exposure (dose) tables for hematoporphyrin derivative photoradiation therapy. *Lasers Surg Med* 1984;4:107-131.
 48. Dougherty TJ: Photosensitization of malignant tumors. *Semin Surg Oncol* 1986;2:24-37.
 49. Chakrabarti P, Orihuela E, Motamedi M, Adesokan A, Cowan D, Warren M: Mechanism of injury of photodynamic therapy (PDT) in the canine prostate: A histopathologic and electron microscopic study. Seventy-fifth Annual Meeting of the South Central Section of the American Urological Association. Vail, Colorado, September 1996. Abstract 80, page 202.